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THE ULTRASTRUCTURAL DAMAGE AND CONSEQUENCES OF PANCREATIC EXOCRINE ENDVENTRICLES IN EXPERIMENTAL ENDOTOXIN SHOCK *

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Abstract

The investigations into the ultrastructural damage of the pancreas in endotoxin shock has so far escaped the attention of researchers. The authors demonstrate in the exocrin cells of the pancreas of dogs, treated of *Escherichia coli* O_{26} endotoxin, such ultrastructural alterations which cannot be observed in case of other organs, after being similarly treated and from which we can draw conclusions concerning the pathomechanism of shock, not made entirely clear until now. It is the most remarkable feature that in the celis no lysosomes resp. Formations referring to some lysosomatic activity are visible; at the same time, there are large vesicles and therein myelin-figures, limited by the outer membrane of mitochondria, in the place of mitochondria. The degradation of mitochondria can, therefore, not be explained with the effect of lysosomal enzymes. The hypothesis of the authors is that the endotoxin getting into the pancreatic cells induces the DNA-RNA systems of the mitochondrium and, consequently, some autolytic ferments are formed and gradually dissolve the proteins of the inner membrane. And the rolledup lipid membranes are arranged into myelin-figures. It may be supposed that during the autolysis of mitochondria vasoactive resp. cell-damaging polypeptides are formed. These shock factors and membrane-toxins, getting into the blood stream, are responsible for the irreversible damage of the shocked organs. - On the basis of our results, the pancreas should be considered as a primary shock-organ.

Key words: pancreas, exocrin ventricles, endotoxin shock, *Escherichia coli*, dog, lysosoma, mitochondrium, myelin-figure.

Introduction

Shock is a complex insufficiency comprising the whole organism. From aetiological point of view, we differentiate between traumatic, haemorrhagic, cardiogenic, anaphylactic and toxic shocks. The endotoxic shocks, elicited by Gram-negative intestinal bacteria, fall within the latter group.

* This paper is dedicated to the centennial anniversary of Prof. AMBRUS ÁBRAHÁM's birth.

The leading clinical symptoms of the endotoxic shock are: the disturbances o: microcirculation and macrocirculation (cardio-vascular insufficiency), coagulopathy, hepatic injury, renal tubules, renocortical necrosis, "shock lungs". The endotoxin shock is - despite the present-day intensive, complex therapy - of a 60 to 80 p.c. letality.

The experimental endotoxin shock can be elicited easily. It is rendered possible by the ultrastructural investigation into the shock organs to study the formation of the irreversible coronary damages, to clear up the causes, pathological processes of shoks. Its importance is considerably high if we approach the opinion of FINE et al. (1959), according to which endotoxins have a part in the pathogenesis of every shock, and even we shouldconsider the endotoxin as the main factor responsible for irreversibility.

Endotoxin contains mor than one kind of toxicants like toxic proteins, N-acetyl-amino-hexuronic acid, lipopolysaccharides (LPS). Its most toxic substances are LPS and among these, as well, the basic units of mean molecular size (3 to 4 million) (BEER et al. 1965).

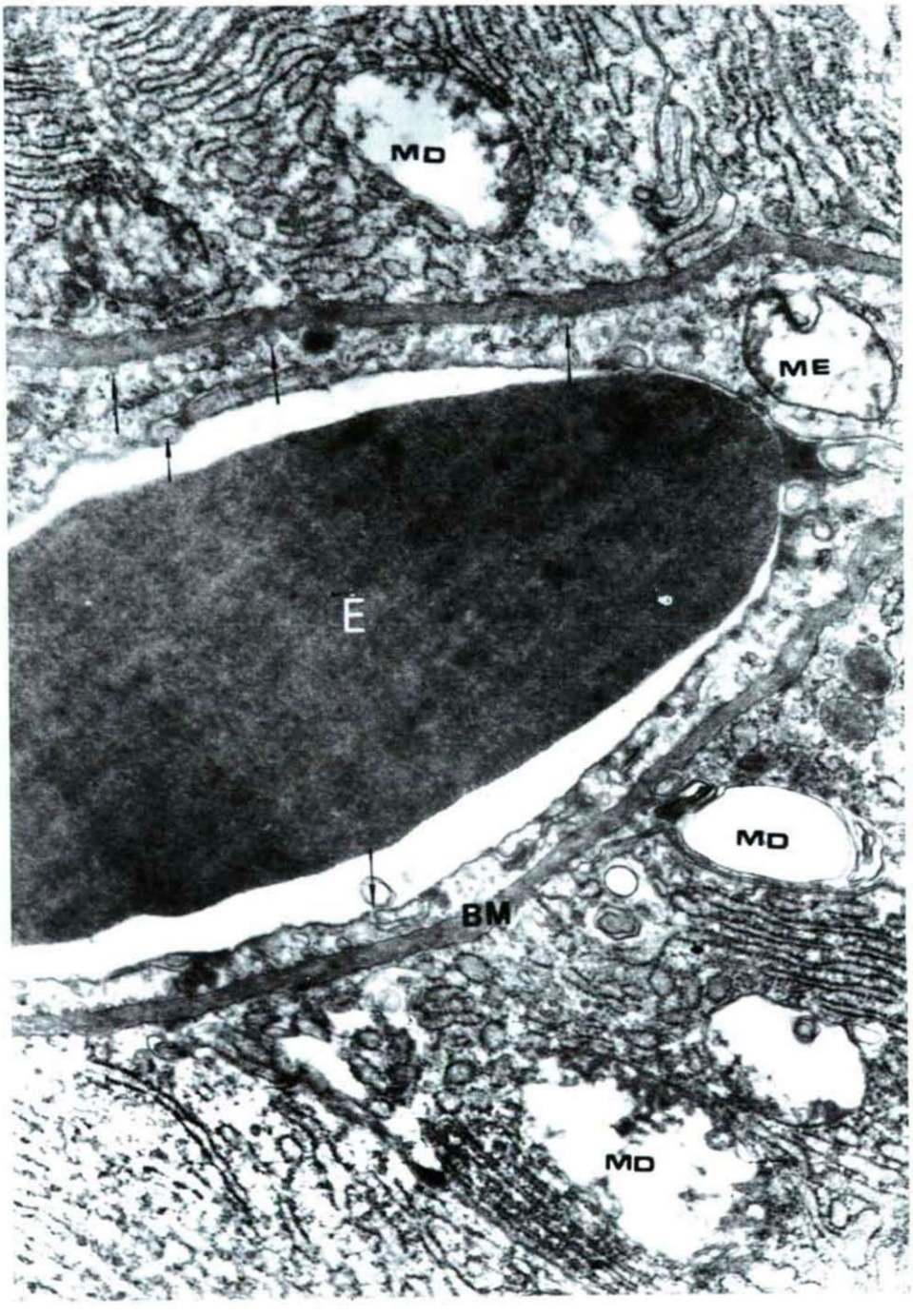
It has long been known, on the basis of experiments performed with fluorescing anti-bodies (AVETIKYAN and KARASIK 1960; ARKADEVA 1963), that toxins get in the cytoplasm through the cell membrane and, after a very short time they can already be demonstrated in the nucleus itself. And even they get over through the membrane of mitochondria, as well. For instance, the filtrate of *Clostridium perfringens*, marked with C_{14}, is exclusively incorporated, according to ELLINGER (1961), in the mitochondria of liver.

The bondage conditions of the receptors of the different cells and endotoxins is not known, as yet. It cannot be said, "which the cell types are to be considered as primary targets" (BERTÓK 1978).

The literature of lightmicroscopical researches, dealing with the effect of endotoxin shocks, is rich. So much the more pitiable are the electronmicroscopical histological data. The mostly investigated "shock-organ" of high priority is the liver (BOLER et al. 1969; MC KAY et al. 1967; DE PALMA et al. 1967; LEVY et al. 1968). The cause of this - demonstrated long ago by BRANDIS et al. (1954); DOERLING et al. (1959) - is probably that two hours after the i.v. injection of the endotoxin, 72.2 p.c. of the injected endotoxin is to be found in the liver.

It is surprising that the researchers have not dealt with the effect of endotoxins, made on the ultrastructure of the pancreas. We consider, therefore, as important to discuss our work, as well as to draw the conclusions from our results.

Fig. 1. Exocrine end-ventricle of a canine pancreas. Capillary (endotoxin). E=erythrocyte; BM=membrana basalis; ME=mitochondrium of the endothelial cell; MD=degraded mitochondrium of the exocrine cell; arrows=pynocytotic and exocytotic vesicles. x22500.


MD
ME
E
MD
BM
MD

Material and methods

Our experimental programme began in the Spring of 1980, in the Veterinary Institute in Miskolc, with dogs. In order to observe the physiological parameters, we canulated in Ketalar-narcosis the aorta through the arteria femoralis and the lower empty vein through the vena femoralis. The endotoxin with *Escherichia coli* O_{26} was introduced in infusion similarly through the vena femoralis.

In the first three dogs, the 100 p.c. lethal dose was titrated. This corresponded to 20 ml/body weight kg endotoxin suspension resp., after determining the dry substance, to 30 mg/body weight kg *E. coli* O_{26} endotoxin quantity.

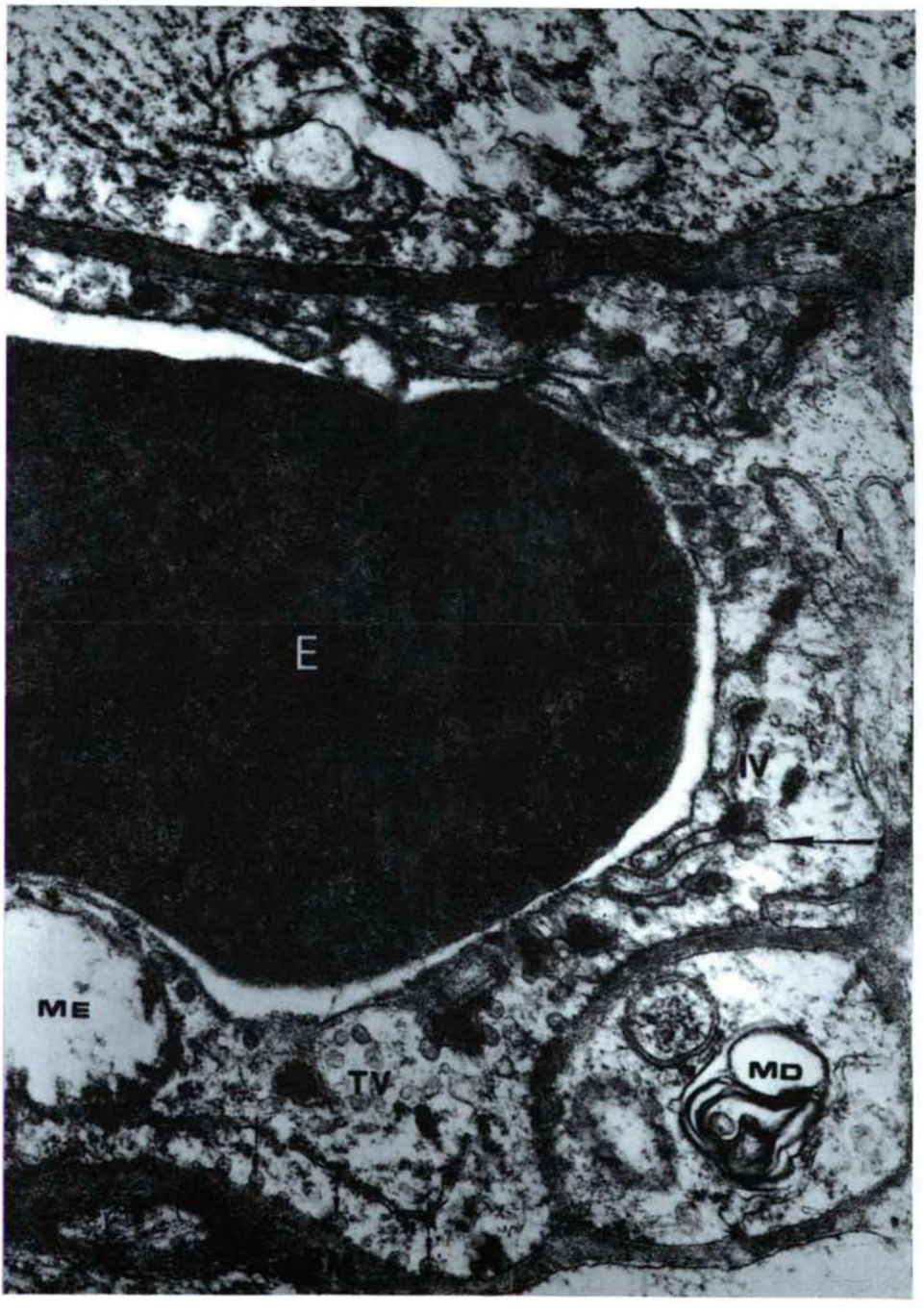
The next occasion, we examined the direct arterial pressure, the central venous pressure, the pulse and respiratory rates on three dogs poisoned with a 100 p.c. lethal dose and on two control dogs each. Then we performed the routine examinations, determined the differences in coagulation and blood gases. After the death of dogs (4 resp. 5 hours), resp. following the over-anaesthesia of control dogs, some substance was taken from the then "usual" organs (liver, kidney, lungs, heart, duodenum) for light- and electron-microscopical investigations. Then we thought of that the entero-bacteria may, by all means, have an effect on the pancreas. Thus, the samples taken from the pancreas were also fixed.

The lightmicroscopical substance was fixed in Bouin, and embedded - after dehydration and treatment with methylbenzoate - in paraffin. The sections were stained with Weigert's haem.-eosin and Mallory. The electronmicroscopical substance was fixed in 2.4 p.c. glutaraldehyd + 0.1 M Na-cacodilate buffer, later in 1 p.c. OsO_4, buffered similarly with Na-cacodilate. The dehydration was performed in alcohol series and acetone. The substance was embedded in araldite. The sections were made in the Department of Zoology of the Eötvös Lóránd University with ultramicrotome Reichert OM U2 and investigated at the same place with an electronmicroscope of Type Tesla BS 500. For supporting our work, we are indebted to reader and head of department Dr. JÁNOS KOVÁCS and for his expert advices of great value, to reader LAJOS KONDICS.

Results

The capillaries of the exorcine end-ventricles of the pancreas show the change, characteristic of the endotoxine effect. In their lumen there are stagnating erythrocytes. The basal membrane of the endothelial cells is disproportionately thick but, in respect of its function, it is rather "loosened". In the loose membrane the pinocytotic vesicles almost reach to one another. Between the plasmic membranes of the adjacent endothelial cells some delated interdigital gaps are visible. The cytoplasm is full of transport vesicles. These stream towards the deep invaginations of the plasmic membrane, facing the vascular lumen, and then evacuate with exocytosis into

Fig. 2. Exocrine end-ventricle of a canine pancreas. Capillary (endotoxin). E=erythrocyte; BM=membrana basalis; TV=transport vesicle; IV=invaginatio; I=interdigital gap; ME=endothelial cell mitochondrium; MD=autolytic mitochondrium of an exocrine cell; arrows=pynocytotic and exocytotic vesicles. x39000.


E
IV
ME
TV
MD

the lumen of the capillary. In the endothelium of the capillary take, therefore, place very intensive metabolic processes. The mitochondria of endothelial cells were, as a consequence of the high-degree hypoxia, degraded, but there are no myelin-figures in their places (Figs. 1. 2.).

As a result of endotoxin, a certain disturbance manifests itself in the secretory processes of cells. This is demonstrated by the difference in form and size between the praezymogenous and zymogenous granules, being a little electron dense and visible in large number in the apical part of cells, as well (Fig. 3.).

It is very characteristic that the ultrastructure of exocrine cells of the pancreas, treated with endotoxin, with the exception of mitochondria, shows no full degradation, even if death comes. On the cell nucleus, no particular changes are to be seen, the cell nucleus is healthy - the pores are a little wider. The Golgi-system is preserved, the granular endoplasmatic reticulum (ER) is only partly damaged. The secretory processes do not come to a standstill. The forms of cell connection (junctions, interdigital connections) are hardly wider (Fig. 4.).

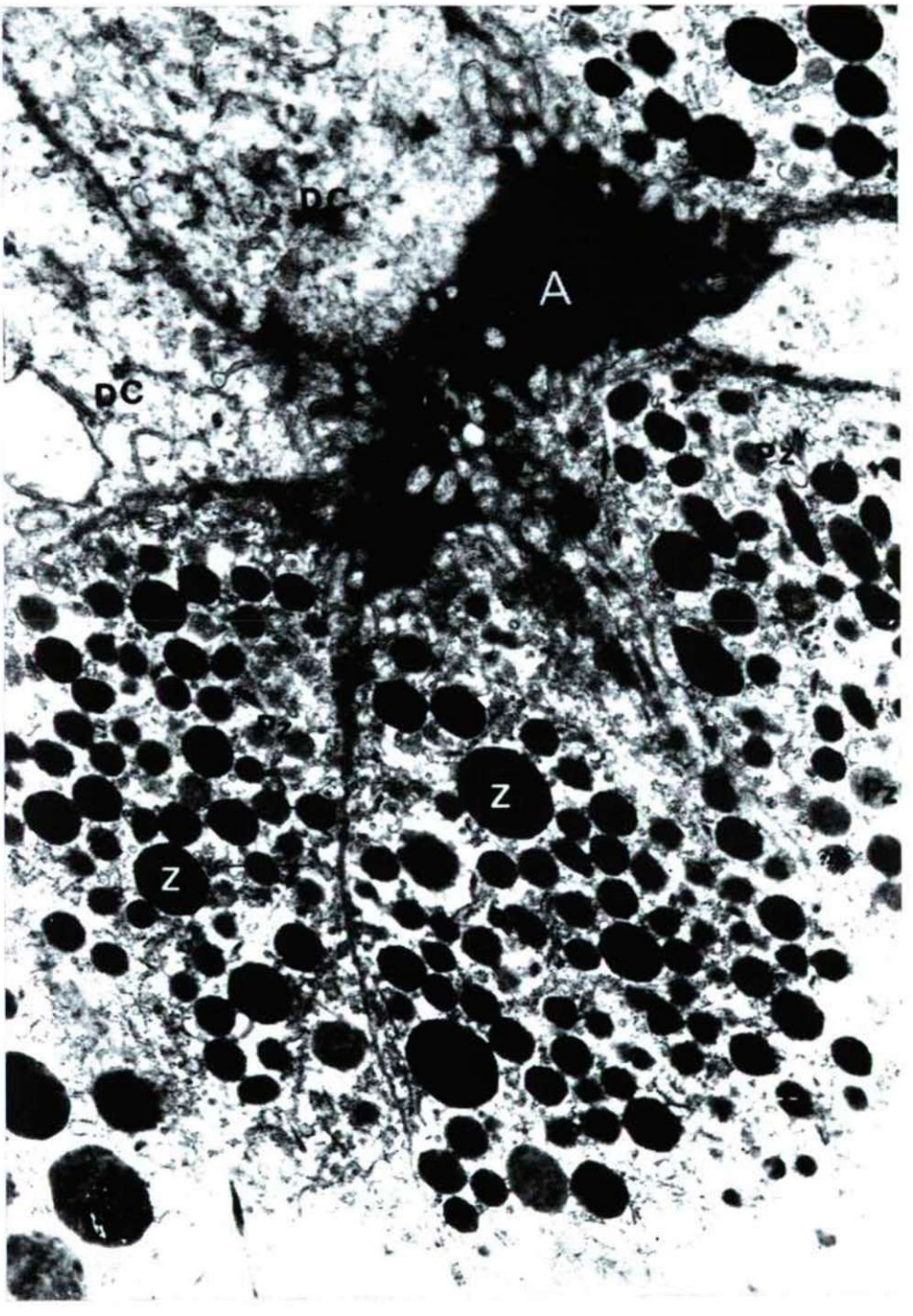
There cannot be observed any primary, secondary lysosome formation. And even, the lack of residual bodies of lysosomal origin refers to that there was no noticeable lysosomal activity in the cells, during the whole endotoxic treatment, either.

The cisterns of the granular endoplasmatic reticulum show the regular laminar picture, which is generally characteristic of the healthy pancreas but - mainly close to mitochondria - they widen out strongly, are vascularized, containing a somewhat electron-dense, finely garnular, lipid-like substance, which may supposedly be LPS (endotoxin), having got in the endoplasmatic system (Figs. 5. 6.).

In cells, apart from rare exceptions, there is no intact mitochondrium! As a result of the substance (endotoxin?), coming out of the cisterns, opened vesicles of the endoplasmatic reticulum, the space between the two membranes grows namely continuously and in the meantime, the dissolution of the cristae of the inner membrane begins. Finally, there remains in the place of the mitochondrium the vesicle, limited by the outer membrane and, inside of it, a huge myelin-figure. (The autolysis of mitochondria, resp. the formation of the myelin figures is clearly shown by Figs. 4, 5, 6 and 7).

Figure 8 is showing the direct contact between the cistern of dilated the endoplasmatic reticulum and the mitochondrium. And in Fig. 9, we can observe the invasion of a huge LPS-drop, accumulated after cracking of the endoplasmatic reticulum in a mitochondrium.

Fig. 3. Exocrine end-ventricle of a canine pancreas (endotoxin). A=acinus; Z=zymogenous granule; Pz=prezymogenous granule; DC=degraded cells. x18000.


DC
A
DC
Z
Z
Z

Discussion

In case of animals in endotoxin shock, the myelin-figures appearing only in the pancreas attrack the attention to the pathomechanism of the endotoxin shock, give an explanation for its course which is more serious than any other kind of shocks, and also for its death rate showing high percent.

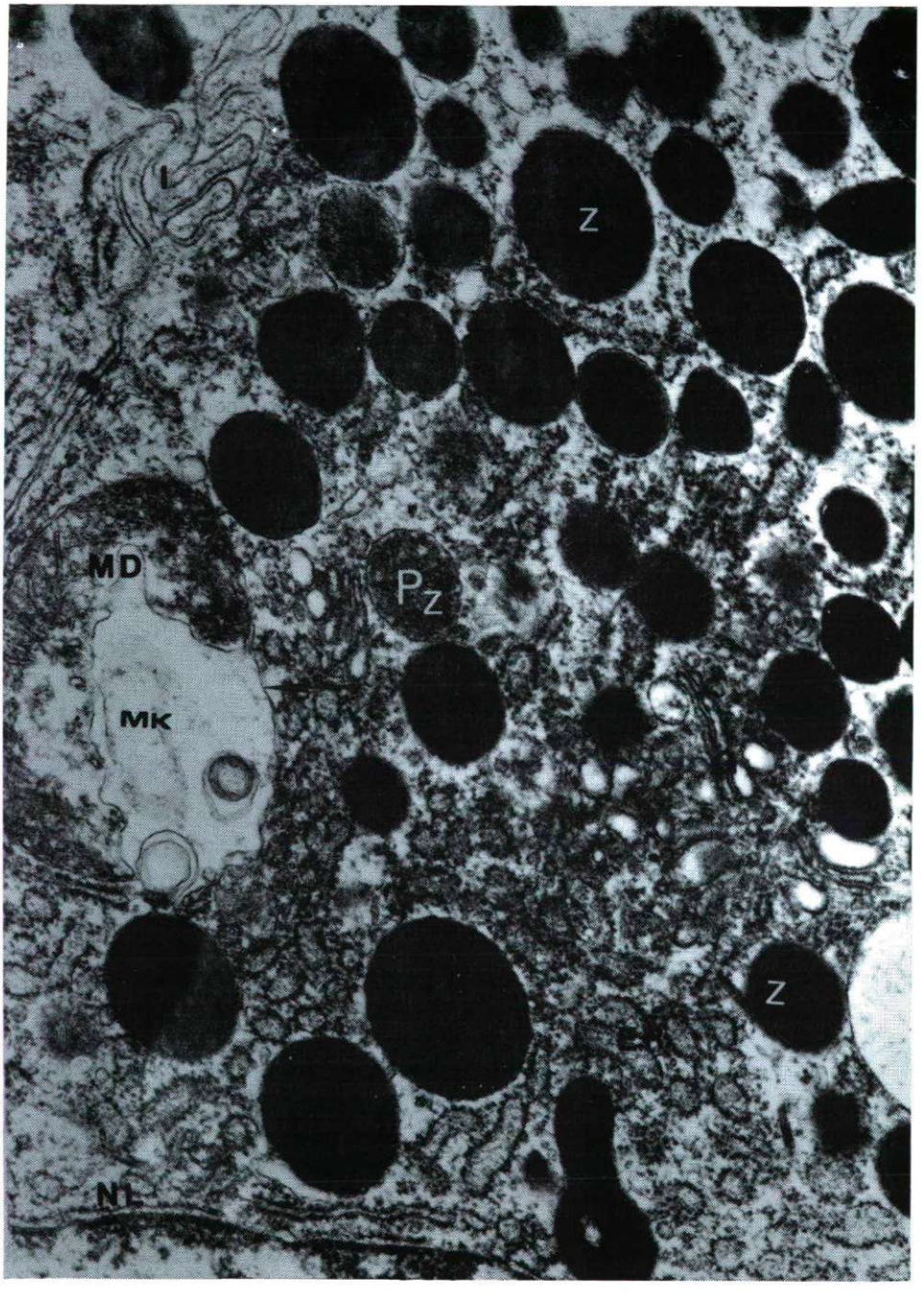
After recognising the effect of the lysosomial enzymes (WEISMANN and THOMAS, 1962, 1964; JANOFF et al., 1962; JANOFF, 1964; DE PALMA et al., 1967; LAPIS, 1974) on the basis of the results of physiological, biochemical and electronmicroscopial examinations a new theory has been found, according to which for the irreversibility of the shocks the lysosomial enzymes activated in the hypoxaemial and acidotic cells are responsible, which in their place of formation, in an autolytical way cause the sinking and at last the death of cell. Among the organs, the most deeply influenced liver, the kidney and the lungs have been considered as primary shock-organs.

The attention has been drawn to the connection between the pathological function of the pancreas and the effect of the endotoxins from the 1970-ies. The works of FINE (1975), FLENKER and LIEHR (1978), LIEHR et al. (1980), SEIFERT (1970), LEFER (1974), GLENN and LEFER (1970) pointed out that a connection has to be searched for between the function of the shocked pancreas, the endotoxaemia and the irreversibility of shocks. FINE (1975) presupposed that in pancreatitis endotoxins can get transmurally from the colon to the circulation and they have biological activity there. It has been confirmed by LIEHR and his colleagues, who could detect the endotoxaemia in pancreatitis by Limulus-test. They found that the extrahepaticus complications, such as clotting troubles, renal insufficiency, lesions of the lungs, and even death, have appeared only in those cases, when the endotoxaemia could have also been detected. FLENKER and collaborators, LIEHR and his colleagues, LEFER, GLENN and LEFER found as the role of pancreas in the process of the development of the irreversibility, that from the shocked pancreas exocrin cells shock-specific mediators (MDF - miocardial depressant factor) could evolve, which could cause the hurt of the heart, the lungs and other organs.

MELA et al. (1970, 1981) mention some kind of "membrane toxins", which are formulated by the participation of mitochondria in endotoxaemia and in tissue hypoxaemia, and hurt the biological membranes.

MORI et al. (1981) examined the effect of lysosomial enzymes with normal rats and also with rats treated with endotoxins. The enzymes given either intravenously or intraperitoneally have not caused haemodynamical changes neither with the normal, nor with those animals which have been treated with a letalic endotoxin dose. Also the combined use of the endotoxin and the lysosomial enzymes have not influenced the

Fig. 4. Exocrine cell of a canine pancreas (endotoxin). I=interdigital gap; Z=zymogenous granule; Pz=prezymogenous granule; MD=degraded mitochondrium; MK=outer ventricle of a mitochondrium; arrow=outer membrane of a mitochondrium; ER=granular endoplasmatic reticulum; NL=nuclear membrane. x39000.


I
Z
MD
Pz
MK
Z
NL

circulation and the mortality of the treated animals. Finally they pointed out: - their experimental data have run counter to that in endotoxin shock the lysosomial enzymes got into the circulation would have had a significant role in the development of the irreversibility of the shock.

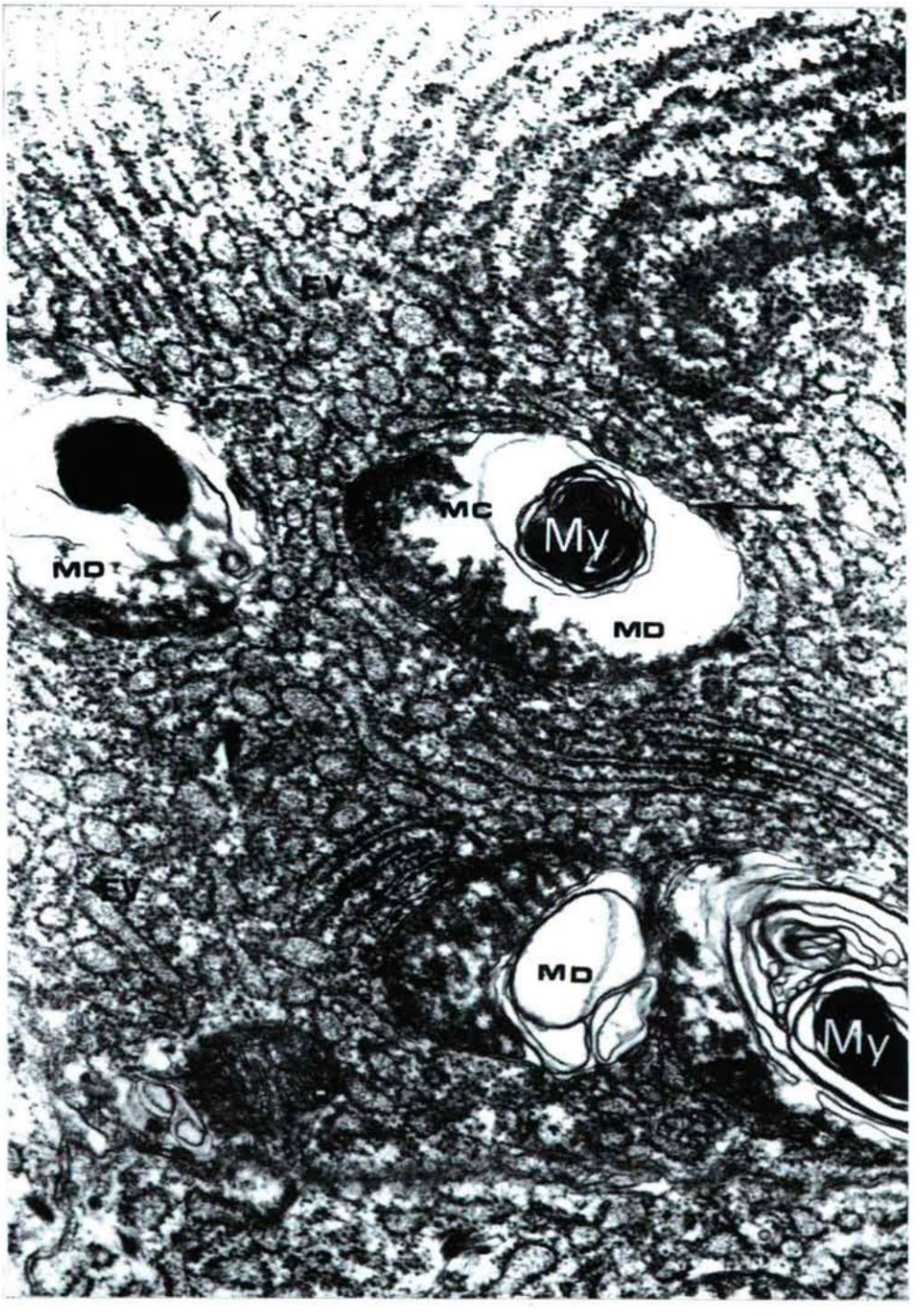
In spite of the connection of clinical importance between the pathological state of the pancreas and the endotoxaemia, we only know the study of NAYYAR et al. (1985) about the ultrastructural lesions of the endotoxin shocked pancreas. The aim of their examination was to study the autophagia in the liver, the kidney and the pancreas of 10-day-old rats. They found that autophag vacuolums were formed in the pancreas as the effect of the endotoxin. These could get into membrane-fusion with the mitochondria, and as a consequence secondary lysosomes bounded by simple membrane and residual bodies were formulated. We have not seen such phenomena in the exocrin cells of the pancreas of neither dogs nor rats. During the formation of the myelin-figures, we have never seen the fusion of the mitochondrium membranes with the membranes of other cell-organells. Moreover it can be clearly observed as the inner membrane moves away from the unhurt outer membrane and after it the cristolysis is beginning.

In our case what is important is the primary change caused by the endotoxin, the conditions of the formation of secondary bodies cannot be seen.

During our experiments, we kept the animals alive generally for 4 hours. Within this period of time the ultrastructure of the pancreas exocrin cells has been scarcely changed with the exception of the mitochondria. We have not met bigger necrosis or focalis described at shocks of other origin. In endotoxin shock in the exocrin cells of the pancreas of dog the inner membrane-system of the mitochondria has been degraded and myelin-figures have been formulated in the bladders bounded by the outer membrane of the mitochondrium. We cannot consider them as growths on the effect of hypoxaemia or acidosis, but they cannot be the result of lysosomial enzyme activation as well, as in the cells the number of the lysosomes does not grow, residual bodies cannot be found there, and on the other hand the free lysosomial enzymes would have hurt the other organelums of the cell. As we have not found such things, we presuppose that the endotoxin directly effects the mitochondria of the pancreas exocrin cells. On its effect the albumin components of the inner membrane-system are resolving an enzymatic way, and the residue lipids become myelin-figures.

The myelin-figures are often considered as artificial results, or simply as precipitates and for their formation mainly the glutaraldehyd fixation is made responsible. The myelin-figures in our pictures can hardly be called precipitations, especially when the gradual cristolysis and the growth of lipid-lamellas can be observed step by step on the mitochondria. On the other hand they were formulated

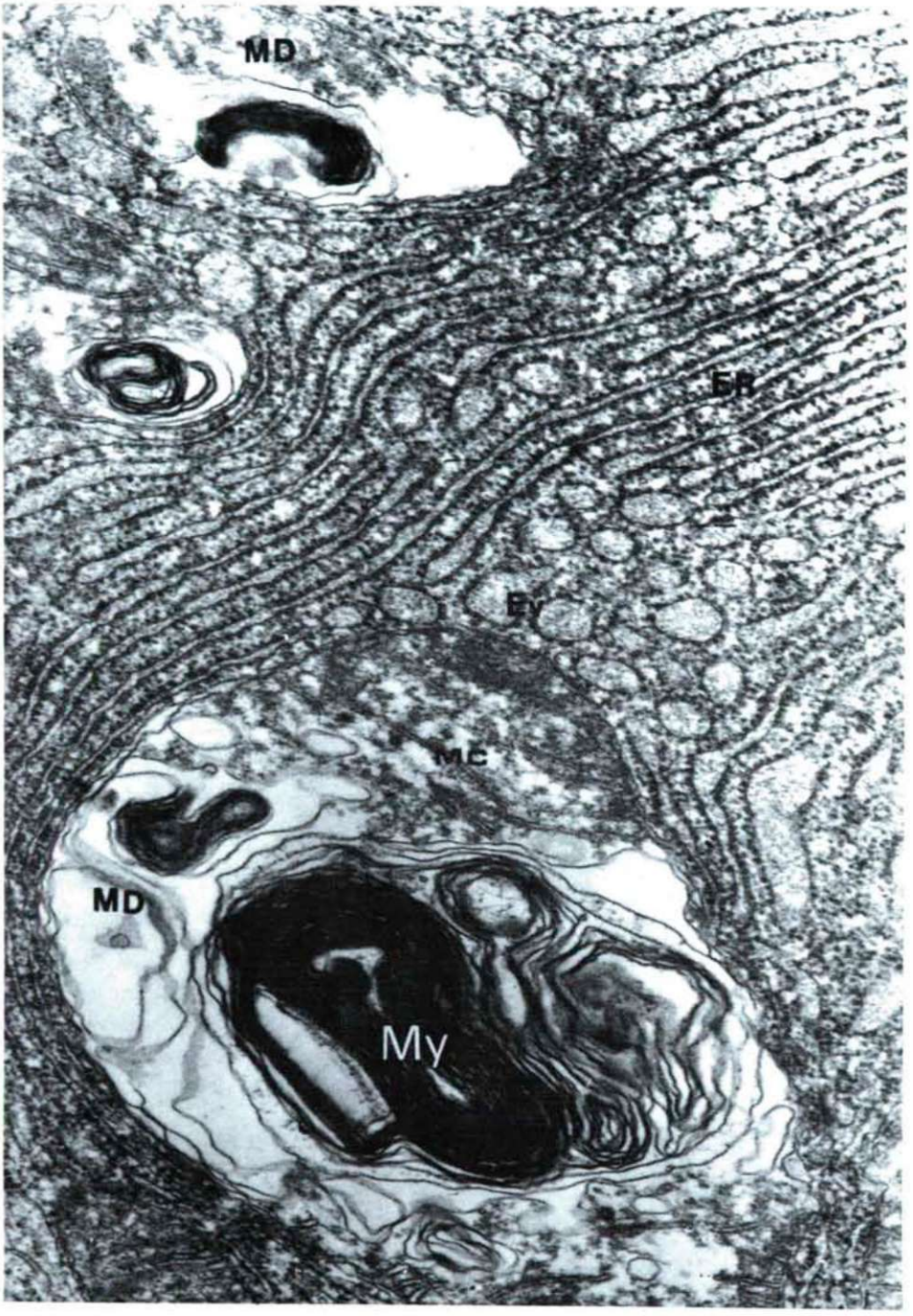
Fig. 5. Exocrine cell of a canine pancreas (endotoxin). EV=vesicularized endoplasmatic reticulum; ER=laminar endoplasmatic reticulum; MD=degrading mitochondrium; MC=crista mitochondrialis; My=myelin-figure; arrow=outer membrane of a mitochondrium. x39000.


EV
MC
My
MD
MD
EV
MD
My

only in the endotoxin shock and only on the mitochondria of the pancreas exocrin cells. They do not appear neither in the cells of other organs, nor in the mitochondria of haemorrhagial shocked pancreas exocrin cells examined as control, though those samples of organs were taken fromdogs and were fixed with glutaraldehyd.

Our hypothesis is supported by the biochemical observations of MCGIVNEY and BRADLEY (1979), BRADLEY (1981) and MELA (1981). BRADLEY presupposes that the endotoxin can get into the cell by endocytosis and the endocytotic vacuolum can merge with a primary lysosome, and also with mitochondrium membrane. So the toxophor can be placed to the receptor in the membrane of the mitochondrium and as its result, within 2-4 hours, the endotoxin can cause an important change in the function of the enzymes of the inner membrane-system. He presupposes that the endotoxin and the lipid-A component simultaneously effect in the inter-membrane space of the mitochondria, in the matrix and on the enzymes found in the inner membrane. MELA caused endotoxaemia and septicaemia in guinea-pigs and dogs, and examined the functions of the mitochondria. He found that the ATP-synthesis, the Ca-transport and the mitochondrial breathing have sufficiently decreased. As he presupposed that these changes can also occur in hypoxaemic state, he repeated the experiments with isolated mitochondria, and he found that the previous processes have decreased more sufficiently. He proved that the trouble in the function of the mitochondria is in direct proportion to the irreversibility and thus to the mortality. Our experiments and electronmicroscopial observations show, that in endotoxin shock the hurt of the pancreas mitochondria can be recognized very soon, after about 30 minutes, so it cannot be expected that the seriously damaged mitochondria will be able to regenerate on the effect of a contingent sudden stop of shock. The destruction of the mitochondria of the pancreas exocrin cells can cause a trouble in the secretional function. The pathological factors, the so-called "shock-factors", getting into the circulation play a part in the serious ultrastructural lesion of other organs and help their irreversible hurt. This kind of function of the endotoxin shocked pancreas has been proved by the animal experiments of SUZUKI et al. (1978). They caused shock on hares by bleeding and partial liver-necrosis (binding of art. hepatica). At a part of the hares the ductus pancreaticus has been bind 10 weeks before, so the pancreas became fibrosical. In the shock caused by partial liver-necrosis, with animals with unhurt pancreas, the perfusion of the brain, heart, the kidney, pancreas, liver and splanchnicus territory has decreased drastically, while in the hares with fibrosical pancreas the circulation of each organ has remained almost normal. The differences indicate that the pancreas has an important role in the development of the shock. From the fact that in case of pancreas fibrosis, in shock, the splanchnical circulation remains normal the authors draw the conclusion that besides the ischaemia other fasctors also play a part in the release of shock-factor from the pancreas. We think that this factor is the endotoxin which got into the splanchnicus circulation and the pancreas.

Fig. 6. Exocrine cell of a canine pancreas (endotoxin). ER=laminar endoplasmatic reticulum; EV=vesicularized endoplasmatic reticulum; MD=degrading mitochondrium; MC=crista mitochondrialis; My=myelin-figure. x39000.


MD
ER
Ly
MC
MD
My

From the cross-checks of the results of our ultrastructural examinations and the data from the above mentioned literature, we can draw the following theoretical and practical conclusions:

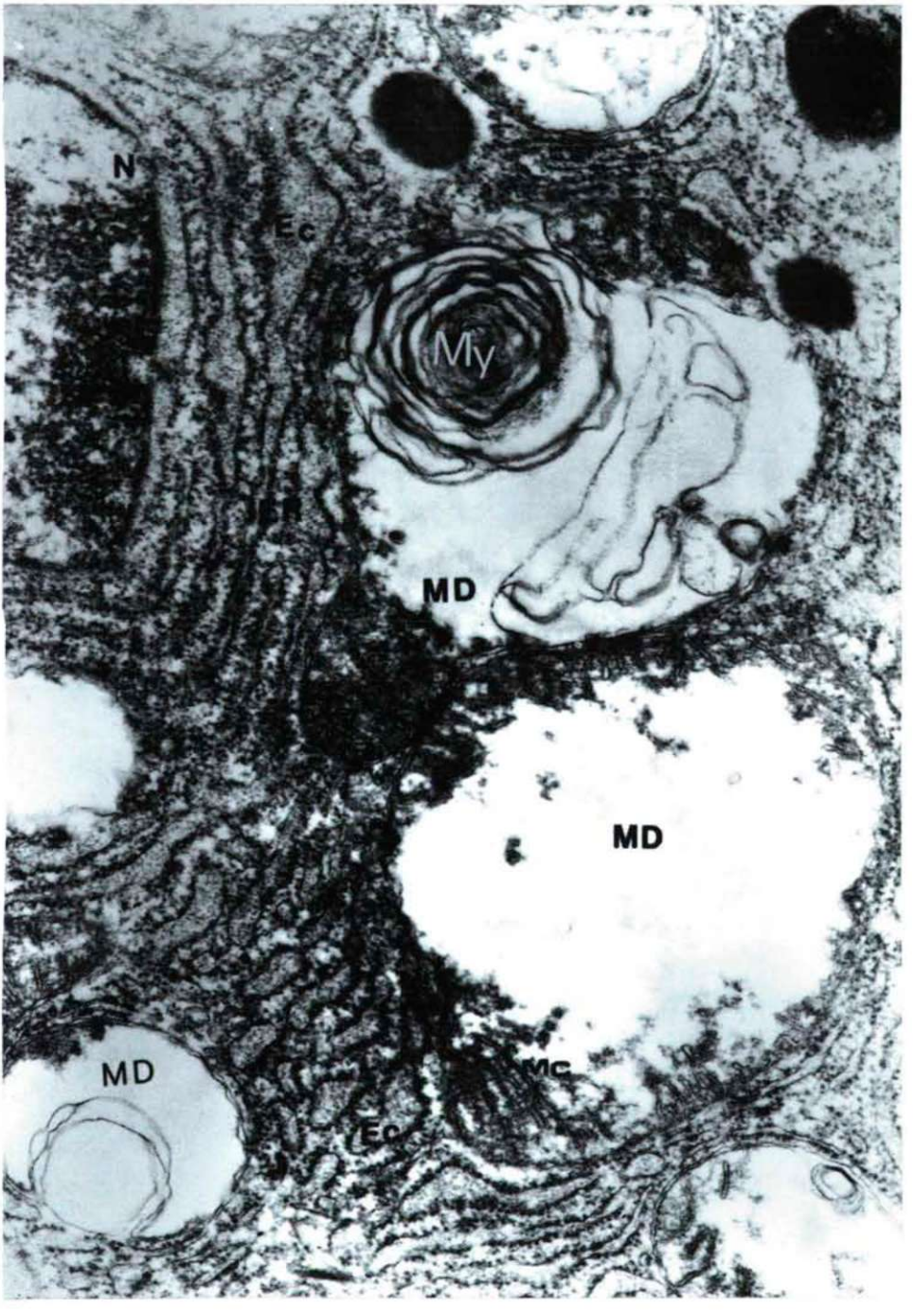
- As the myelin-figures appear only in the pancreas exocrin cells, we could use the pancreas exocrin cells as a model for the evaluation of the endotoxin effect found in other organs.
- We used them as a model for proving the differences in the pathomechanism of the shocks and on its basis proving the direct cell-damaging role of the endotoxins.
- We could examine the effectiveness of different therapeutic processes on sub-cellular level.
- The formation of the myelin-figures, the lack of lysosomial residue bodies prove that it is not the lysosomial enzyme which is responsible for thi high irreversibility of the endotoxin shock, but the endotoxin is, and the processes indicated by the endotoxin.
- The pancreas is a primary shock-organ, moreover, on the basis of the myelin-figures formulated only in the pancreas exocrin cells and its simultaneous patho-physiological processes it can be presupposed that the membranes bounding the mitochondria of the pancreas exocrin cells are the "target" membranes of the endotoxin.
- In agreement with the opinion of many authors, according to which during the development of the myelin-figures, different membrane destructive factors, membrane toxins, opioids, complement activators etc. are formulated, which can effect the different organs, and the endotoxin and the pancreas may have an effect on the final evolvement of every kind of shocks.
- The anoxia, acidosis, formulated by the sympatho-adrenalis system, and as its result the destructive effect of the lysosomial enzyme-system on the organs definitely play a part to a certain extent in the pathomechanism of the endotoxin shock, but from the myelin-figures appearing in the pancreas exocrin cells in the very early phase of the endotoxin shock we can draw the conclusion that in the pathophysiological processes of the endotoxin shock the pancreas has a determinal role.

Fig. 7. Exocrine cell of a canine pancreas (endotoxin). ER=laminar endoplasmatic reticulum; Ec=delated cistern; MD=degrading mitochondria; MC=crista mitochondrialis; N=nucleus. x39000.

Next two pages:

Fig. 8. Exocrine cell of a canine pancreas (endotoxin). BM=membrana basalis; ER=laminar endoplasmatic reticulum; MD=degrading mitochondrium; Z=zymogenous granule; My=myelin-figure; arrow=direct contact between delated cistern and the mitochondrium. x39000.

Fig. 9. Exocrine cell of a canine pancreas (endotoxin). MD=degrading mitochondria; MC=crista mitochondrialis; LPS=lipopolysaccharid drop?; ER=endoplasmatic reticulum; EF=cracked endoplasmatic reticulum; N=nucleus; NL=nuclear membrane. x39000.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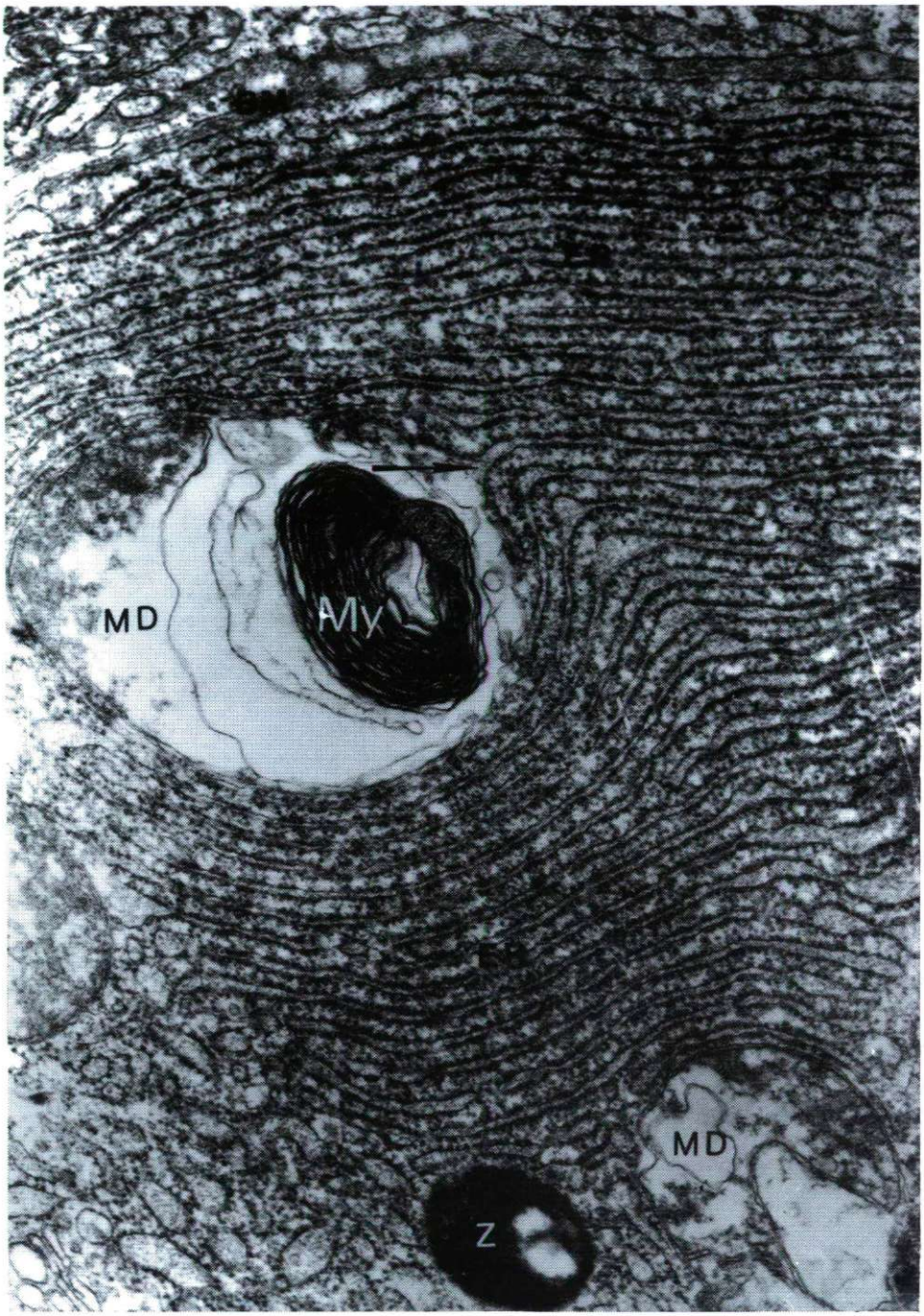
MD
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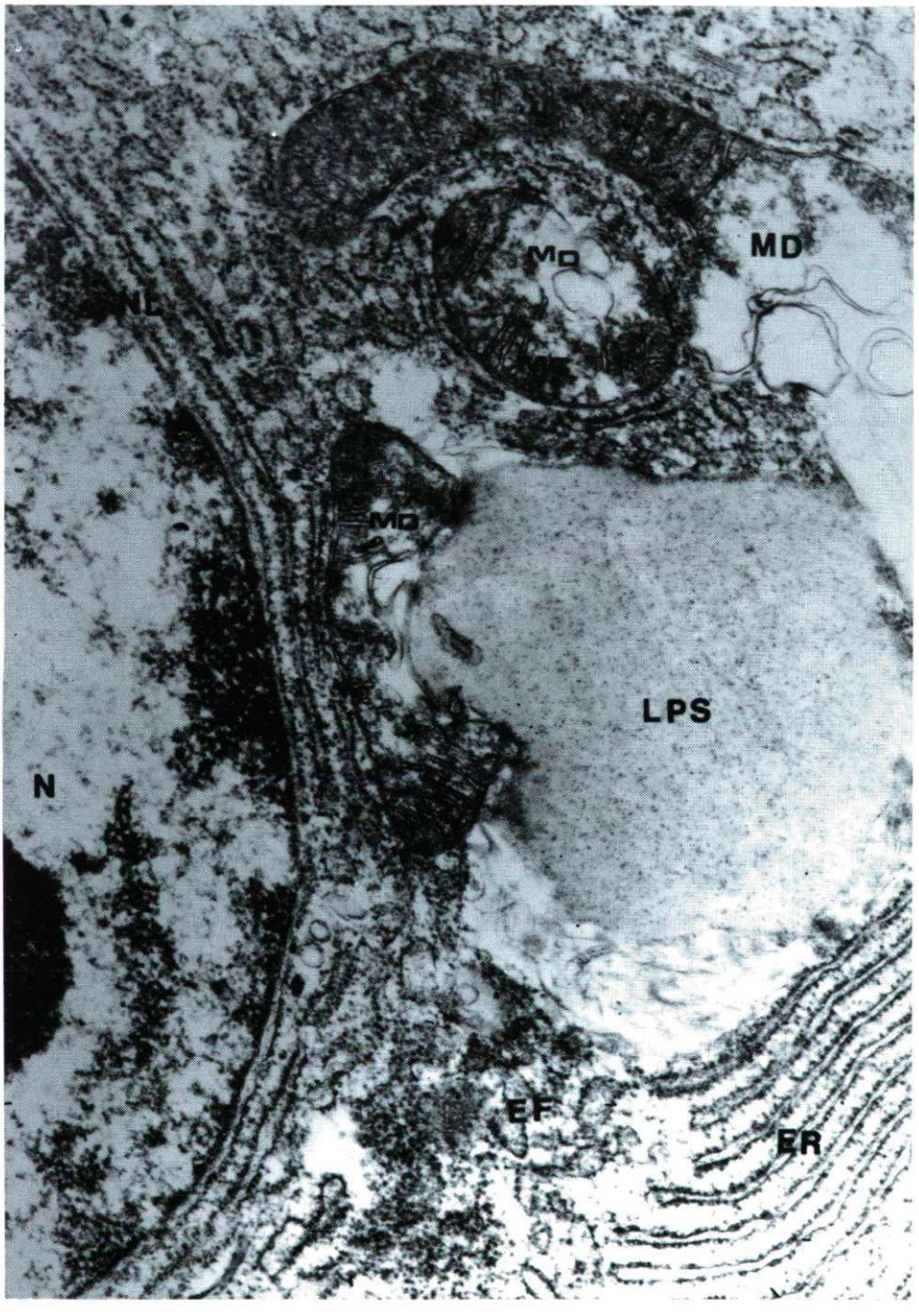
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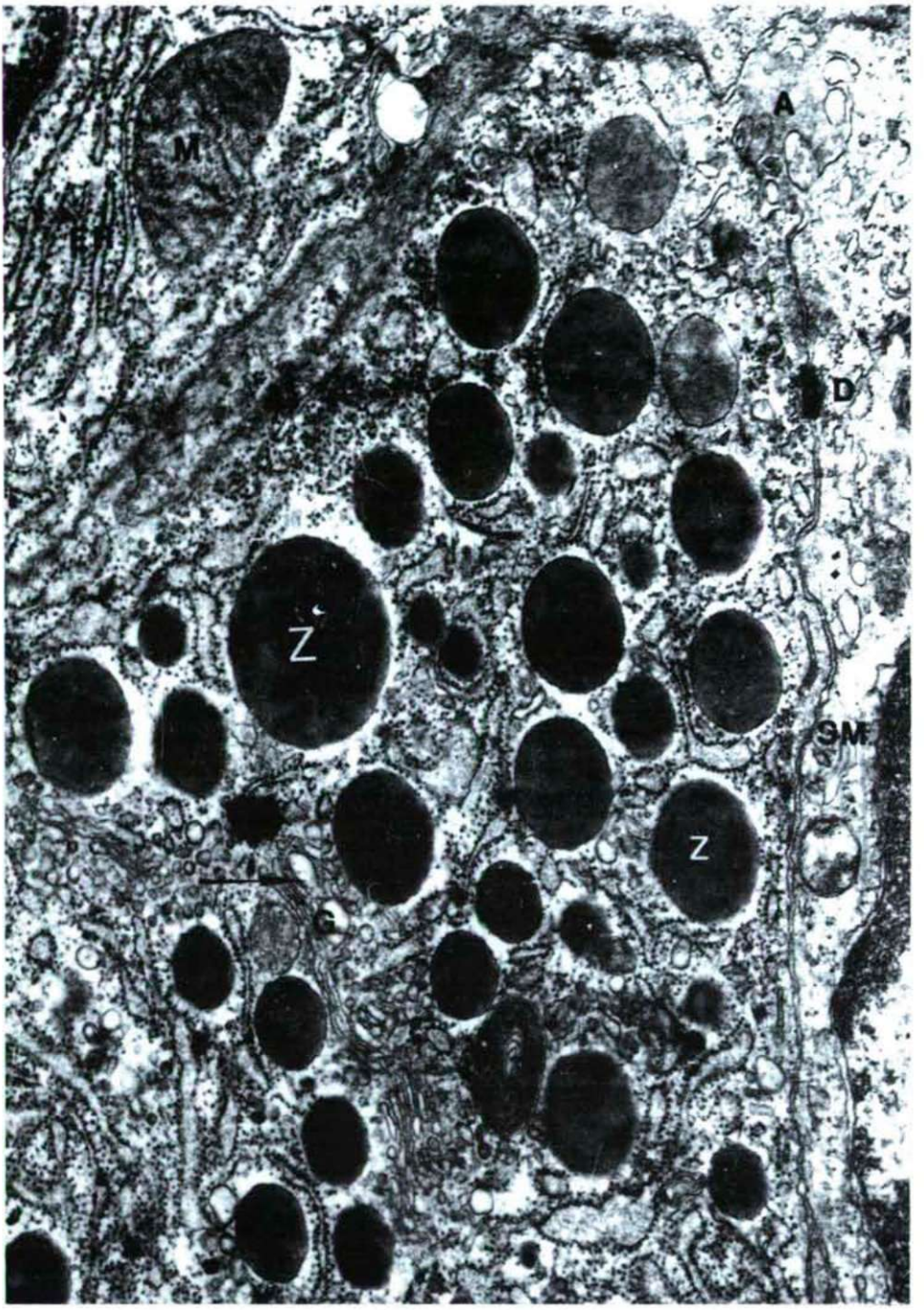
M
A
D
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SM
Z

Fig. 10. Exocrine cell of a canine pancreas (control). A=acinus; M=mitochondrium; ER=granular endoplasmatic reticulum; G=Golgi-system; arrow=Golgi vesicle; Z=zymogenous granule; D=desmosoma; SM=cell membrane. x22500.

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